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Attestation

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Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécifiée à la page suivante.

Patentanmeldung Nr.

Patent application No. Demande de brevet nº

02100662.2



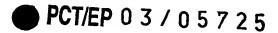
Der Präsident des Europälschen Patentamts; Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets

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### Blatt 2 der Bescheinigung Sheet 2 of the certificate Page 2 de l'attestation

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Anmelder: Applicant(s): Demandeur(s):

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Improved method for the treatment of shredded cheese with a polyene antifungal compound

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## IMPROVED METHOD FOR THE TREATMENT OF SHREDDED CHEESE WITH A POLYENE ANTIFUNGAL COMPOUND

The present invention relates to a method for the treatment of shredded cheese with a polyene antifungal compound.

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The prevention of mould growth is an important topic to the food, feed and agricultural industry, especially in the food industry. Fungal spoilage can lead to serious economic losses. Some foods can be considered as a good substrate for fungal growth. Cheese is an example of such a product. Apart from the negative appearance of fungal growth on cheese, fungal spoilage is also a health risk. Some mould species produce mycotoxins, which may penetrate into the product (J.C. Frisvad & U. Thane; "Mycotoxin production by food-borne fungi" in Introduction to food-borne fungi, 4<sup>th</sup> edn. (ed. R.A. Samson et al.), 1995, 251-260). Therefore, superficial removal of moulds gives no guarantee of safety to the consumer.

For more than 30 years, natarrycin has been used to prevent growth of moulds and yeasts on cheeses and sausages. Natarrycin is on the market under the brand name of Delvocid®, a powder composition containing 50% (w/w) of natarrycin.

Food products can be treated with natamycin in different ways. Natamycin can for example be added to the polymer dispersion that is applied to the cheese rind as a coating (C.B.G. Daamen & G. van den Berg "Prevention of mould growth on cheese by means of natamycin" Voedingsmiddelentechnologie, 1985, 18 (2), 26-29)

Alternatively, food products such as cheeses and sausages can be treated by dipping or spraying with a suspension of natamycin in water (C.B.G. Daamen & G. van den Berg "Prevention of mould growth on cheese by means of natamycin" Voedingsmiddelentechnologie, 1985, 18 (2), 26-29; H.A. Morris & H.B. Castberg "Control of surface growth on blue cheese using pimaricin" Cultured Dairy Products Journal,1980, 15 (2), 21-23; P. Baldini, F. Palmia, R.G. Raczynski, M. Campanini, "Use of pimaricin for preventing mould growth on Italian cured meat products", Industria Conserve, 1979, 54 (4), 305-307; R.A. Holley, "Prevention of surface mould growth on Italian dry sausage by natamycin and potassium sorbate", Appl. Environ. Microbiol., 1981, 41 (2).

Spraying of a suspension of natamycin can be applied for the treatment of shredded cheese, a cheese product which is very sensitive to fungal spoilage. The production of shredded cheese is as follows: cheese, e.g. Mozarella, pizza cheese, or Cheddar is shredded and conveyed to a revolving tumbler. An anti-caking agent, for example cellulose (microcrystalline or powdered), starch or modified starch is metered onto the cheese in the first part of the tumbler. Cellulose forms a film around the cheese particles which prevents caking of the shredded cheese. At the end of the tumbler a suspension of natamycin (e.g. Delvocid®) is sprayed onto the shredded cheese. The spray nozzles deliver a fine spray or mist of the fungicide onto the cheese. Finally the cheese empties onto a conveyor to be transported to the filling equipment.

The natamycin suspension is prepared by mixing Delvocid® and water, mostly at the ratio from 2 to 10 grams of Delvocid® to 1 litre of water. The suspension is stored in a liquid container. To prevent sedimentation of the suspension a small amount of the suspension, which is pumped under pressure to spray nozzles, is recirculated to the liquid container via a jet agitator. Alternatively, a stirring device can be used to prevent sedimentation. Mostly approximately 6 to 8 litres of Delvocid® suspension is sprayed onto 1000 kg of cheese. Mostly this treatment is effective to prevent fungal spoilage.

The spraying or showering of aqueous suspensions on cheeses using nozzles is an effective way of coating the cheeses with the antifungal compounds. In general for spraying of complete cheeses a less fine spray is used. In general this spray or shower will be focused or directed onto the cheese. However for spraying of shredded cheese, the suspension should be added as a fine spray or mist. This spraying method gives often rise to problems such as the clogging of the spraying nozzles.

It now has been found that protection against the development of moulds and yeasts shredded cheese is obtained in a method for the treatment shredded cheese with a composition comprising a polyene antifungal compound, whereby the composition comprises a polyene antifungal agent, a thickening agent and water. Surprisingly it has been found that the thickening agent prevents the clogging of the nozzles. Moreover the aqueous composition could be pumped under pressure to the nozzles without problems which could have been expected due to the expected increase of viscosity of the

aqueous composition of the invention compared to an aqueous composition without thickening agent. Especially xanthan gives very good results.

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The polyene antifungal compounds used in the composition of the invention are preferably one or more of natamycin, nystatin, lucensomycin or amphotericin B. The preferred polyene compound is natamycin. In the preparation of compositions of the invention, one or more polyene antifungal compounds may be used or pre-prepared compositions containing such antifungal components may be used. An example of such a preparation is commercially available powder composition sold under the trade mark Delvocid® which contains 50% (w/w) natamycin.

The concentration of polyene fungicide in the aqueous composition will generally be from 0.1 to 20 g/l, more preferably from 0.5 to 8 g/l and most preferably from 1 to 5 g/l.

In compositions of the invention the preferred thickening agents include all thickening agents known in the art for use in food products, preferably the thickening agents are gum, more preferably xantan gum and/or gellan gum.

The one or more thickening agents in the aqueous composition are preferably present in an amount of 0.5 to 50 g/l, more preferably from 0.5 to 20 g/l and most preferably from 1 to 5 g/l.

In some embodiments it has been found that no buffer is needed for the suspension. In general the pH of the suspension will be between 3-10, preferably the pH will be between 6-9, more preferably between 6.2-8.5 for the optimal natamycin addition.

Preferably also an anticaking agent is added to the shredded cheese. The anticaking agent preferably is a dry powder composition, which is added separately from the aqueous composition. In general 0.2 to 3% w/w of anticaking agent (on shredded cheese) preferably between 1.5 and 2.5%w/w.

The steps of adding the dry powder composition and the aqueous composition may be performed in any order. Preferably, the dry powder composition is added before the aqueous composition is added.

The amount of the aqueous composition added to the shredded cheese may be between 0.01% and 5% (v/w), preferably said amount is between 0.5% and 2% (v/w).

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In a preferred embodiment, the aqueous composition as used in the method according to the present invention is an aqueous solution or an aqueous dispersion such as an emulsion or suspension.

The optimal final concentration of natamycin added according to the method of the present invention, may vary with the type of cheese used as shredded cheese or the kind of shredded used. To prevent fungal growth on shredded cheese, said optimal final concentration may be between 2 and 40 ppm natamycin, preferably said concentration is between 3 and 20 ppm natamycin, more preferably said concentration is between 4 and 10 ppm natamycin.

Any anti-caking agent may be used. Examples of suitable anti-caking agents are microcrystalline or powdered cellulose, starch, modified starch, sodium-, magnesium-, potassium-, and zinc silicate, silicium-dioxide, kaolin, talc, potassium and magnesium carbonate, phosphates (di, tri and polyphosphates including sodium, potassium and calcium salts). Also commercial products containing cellulose, such as Keycel or Floam® may be used as anti-caking agent. Further, any combination of anti-caking agents may be used.

The aqueous composition comprising an antifungal compound, e.g. natamycin, may be prepared by mixing a dry formulation, e.g. Delvocid®, in water using well known methods. Preferably a natamycin suspension for spraying is prepared by using a stock suspension of natamycin as described in EP 0 678 241. This stock suspension is a chemically, physically and microbially stable concentrated suspension of natamycin, which provides a convenient stock for the easy and reproducable dosage of a fungicide to food, feed and agricultural products. Apart from natamycin, the suspension comprises a thickening agent, for example xanthan. The concentration of natamycin in the stock suspension may be as high as 40% (w/w), however also stock suspensions with lower concentrations of natamycin can be used. An example of such a stock suspension is the commercial product Delvocid®-Sol. This product comprises at least 50% (w/w) of natamycin; for example 50 to 54% (w/w) of natamycin, 2% (w/w) of xanthan and 44 to 48% (w/w) of lactose. In the case of shredded cheese the stock suspension is diluted with water to the final concentration required for the treatment. This dilution step can be best executed just before adding the natamycin suspension to the shredded cheese.

In case the polyene antifungal compound is natamycin, also complexes of natamycin (e.g. with proteins) and salts of natamycin (e.g. the calcium salt) may be used in the method according to the present invention. Further, the dry and aqueous compositions may also contain components, which are already present in a natamycin preparation. For example, when Delvocid-Instant® or Natamax® is used, lactose will also be present in the final composition.

The following Examples are for illustrative purposes only, and are not to be construed as being limitative to the invention.

10 Examples

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Cheddar cheese was shredded using well known methods and conveyed to a revolving tumbler. Microcrystalline cellulose is metered onto the shredded cheese in the first part of the tumbler to be thoroughly mixed into the shredded cheese. At the other side of the tumbler, extending about 30 – 60 cm into the tumbler, spray nozzles are situated (S.S. Fogger Tip Assembly #22624-2-80: 80° angle, 2 gal. (8 liter) per hour at 80 PSI (5.3 bar, purchased from Spraying Systems Co. PO Box 95584 Chicago IL 60694). The nozzles are able to deliver a fine spray of mist of one of the following natamycin formulations. Two experiments were carried out using either formulation A (reference) or formulation B (present invention).

- A) A suspension of Delvocid®, available by DSM Food Specialties USA, by mixing 10 grams of Delvocid® with 4 liters of tapwater.
- 25 B) A suspension of Delvocid® and xanthan by mixing 10 grams of Delvocid® with 4 liters of tapwater together with 3 grams Keltrol® RD, available by NutraSweet Kelco Company.

Both suspensions are sprayed on shredded cheese with a flow of 10 liters per hour.

30 In time the spray characteristics are followed during a 2 shifts operation, 16 hours per day.

During the tests it shows that the nozzles could be used with suspension A during 1-6 hours operation, after this period the nozzles were completely blocked by clogging. The nozzles needed to be cleaned completely before further operation could be possible. Using suspension B results in a smoothly operation during 16 hours without any problem such as clogging.

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#### **CLAIMS**

A method for the treatment of shredded cheese with an aqueous composition
 whereby the aqueous composing comprises a polyene antifungal compound and a thickening agent.

- 2. A method according to claim 1, wherein the polyene antifungal compound is natamycin.
- 3. A method according to claim 1 or 2, wherein further a dry powder composition comprising an anticaking agent is added to the shredded cheese.
- 4. A method according to claim 3, wherein the anticaking agent is cellulose or starch or modified starch.
  - 5. A method according to claim 1-4, wherein the thickening agent is xanthan.

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# IMPROVED METHOD FOR THE TREATMENT OF SHREDDED CHEESE WITH A POLYENE ANTIFUNGAL COMPOUND

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#### **Abstract**

A method for the treatment of shredded cheese with an aqueous composition

whereby the aqueous composing comprises polyene antifungal compound and a thickening agent.